

***Pisum sativum* L. 'Eso': Metabolic profiling of yellow seeds to define the optimal harvest time.**

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Abstract: Yellow pea (*Pisum sativum* L. 'Eso', sin. *Lathyrus oleraceus* Lam. (YP)) is an annual herbaceous plant that belongs to the Fabaceae family. Peas, along with other legumes, are an excellent source of proteins and essential amino acids; the yellow variety is known for maintaining a good protein profile even if subjected to industrial processing. However, the presence of antinutrients, such as phytates and oligosaccharides, limits its consumption as a fresh legume addressing its use as a source of isolated proteins or for animal feed. The aim of the study is to evaluate the changes in the entire phytochemical profile of YP seeds as a function of the harvest time. YPs harvested at about 40, 50, 60 and 70 days from sowing were examined by high-resolution NMR spectroscopy employing ¹H-NMR, ¹H-¹H TOCSY and ¹H-¹³C HSQC. 40 molecular species have been identified and quantified; it was observed that there is a monotonous decrease in amino acids, carbohydrates, and secondary metabolites as a function of time. Antinutrients levels increased, but only in later sampling times. This study identified the optimal harvest time for yellow peas "Eso" in fortieth day from sowing adding new information about the best nutritional outcome for humans.

Keywords: Yellow peas, NMR-based Metabolic Profile, Harvest Time

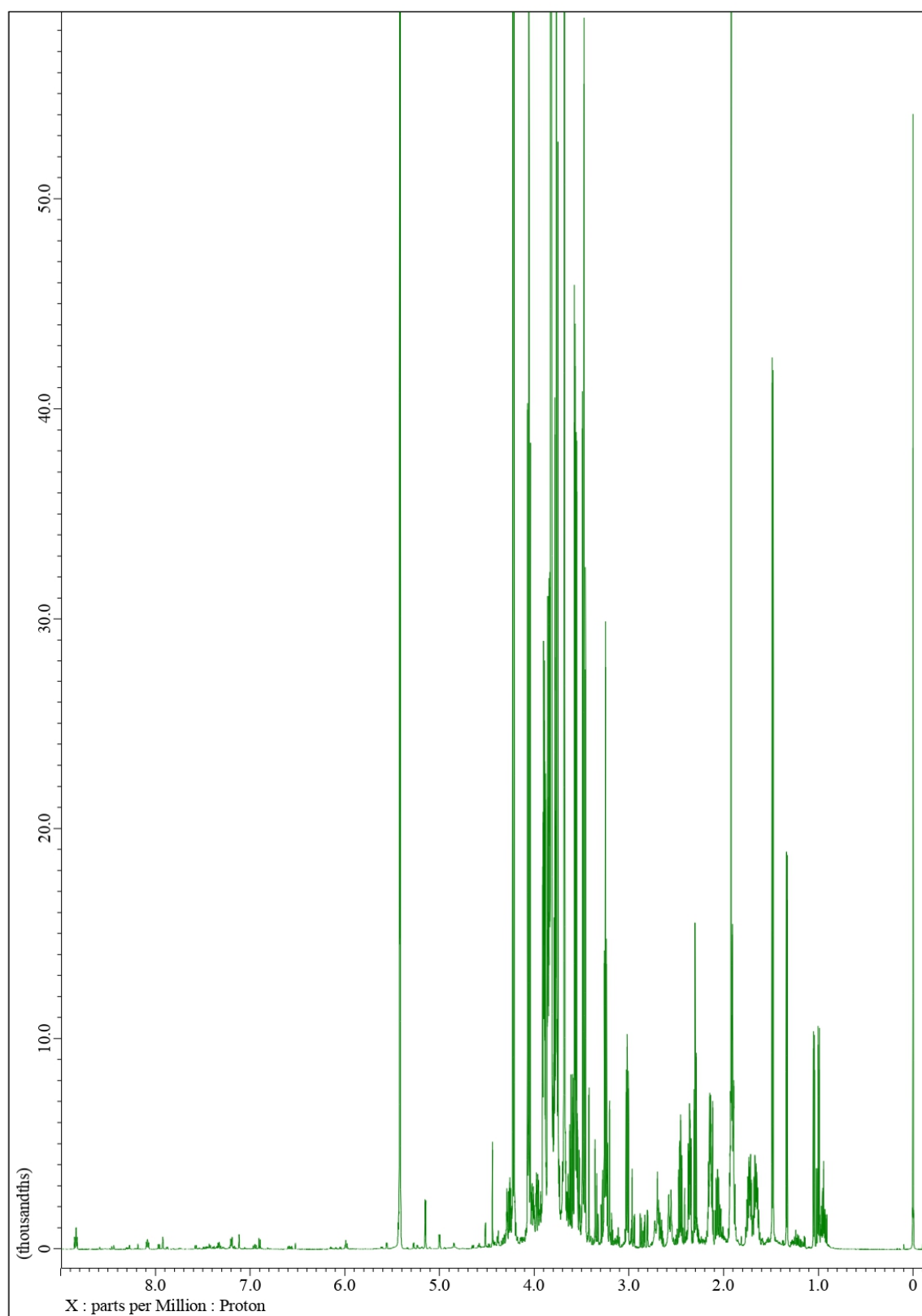


Figure S1: ^1H Spectrum of yellow pea hydroalcoholic extract.

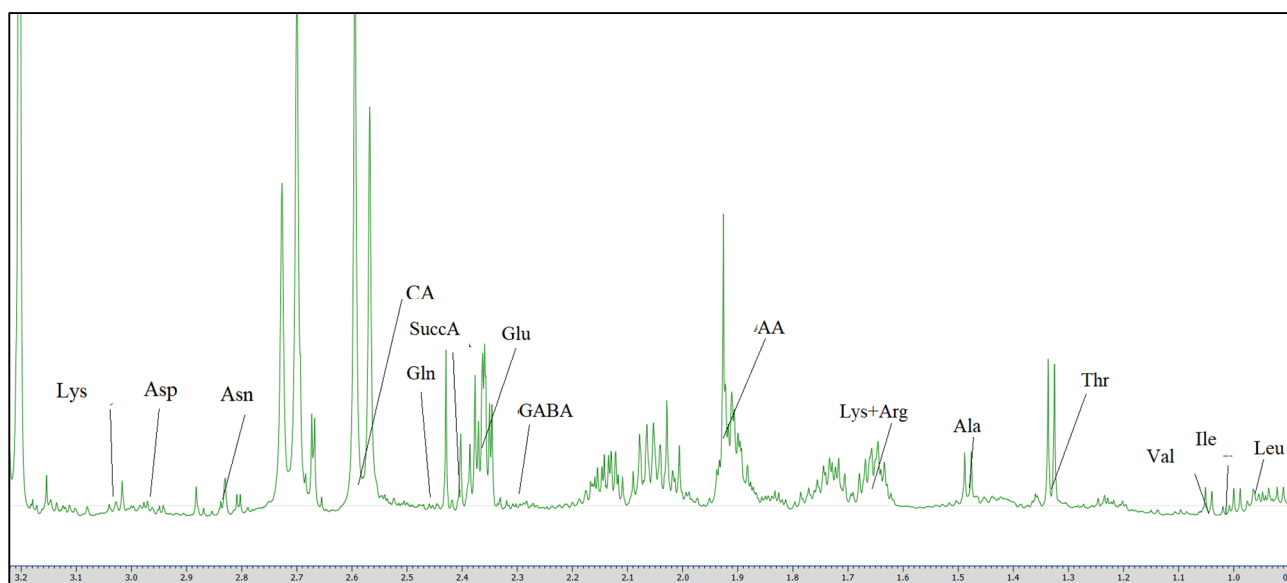


Figure S2: ^1H Spectrum of yellow pea hydroalcoholic extract, 0.9-3.2 ppm. Molecule abbreviations are reported in Table S1.

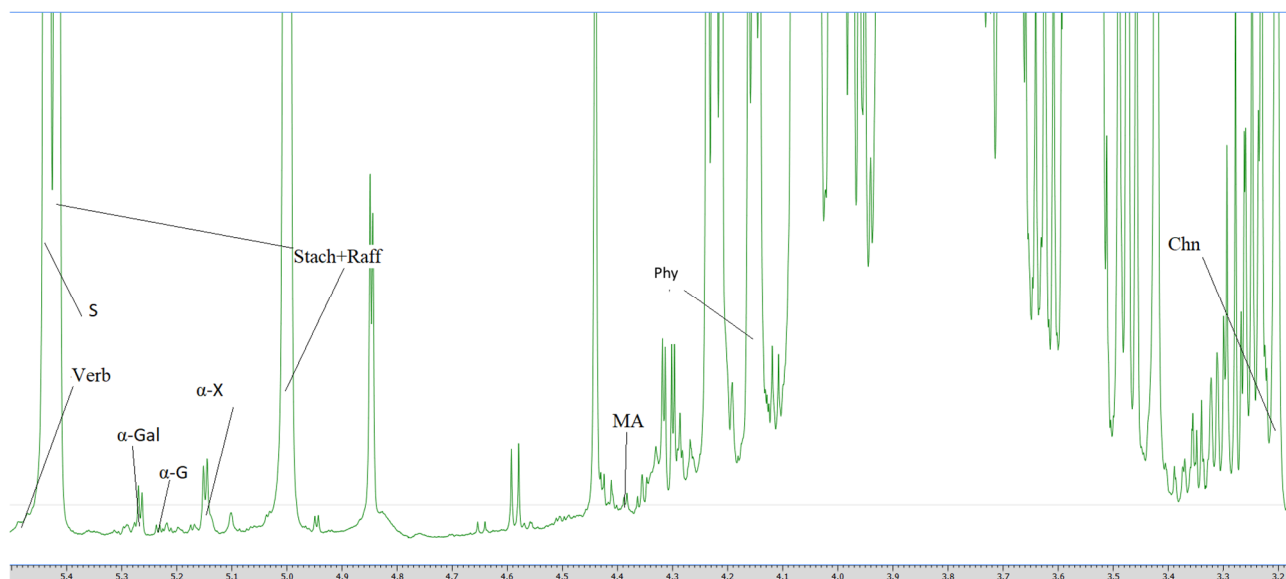


Figure S3: ^1H Spectrum of yellow pea hydroalcoholic extract, 3.2-5.5 ppm. Molecule abbreviations are reported in Table S1.

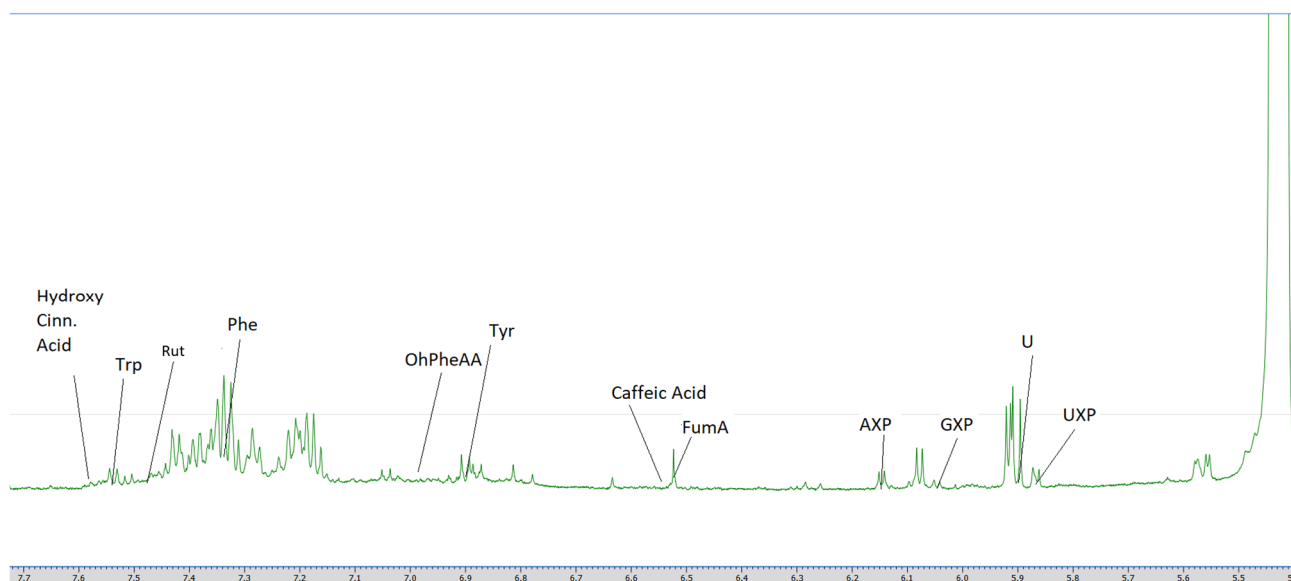


Figure S4: ^1H Spectrum of yellow pea hydroalcoholic extract, 5.5-7.7 ppm. Molecule abbreviations are reported in Table S1.

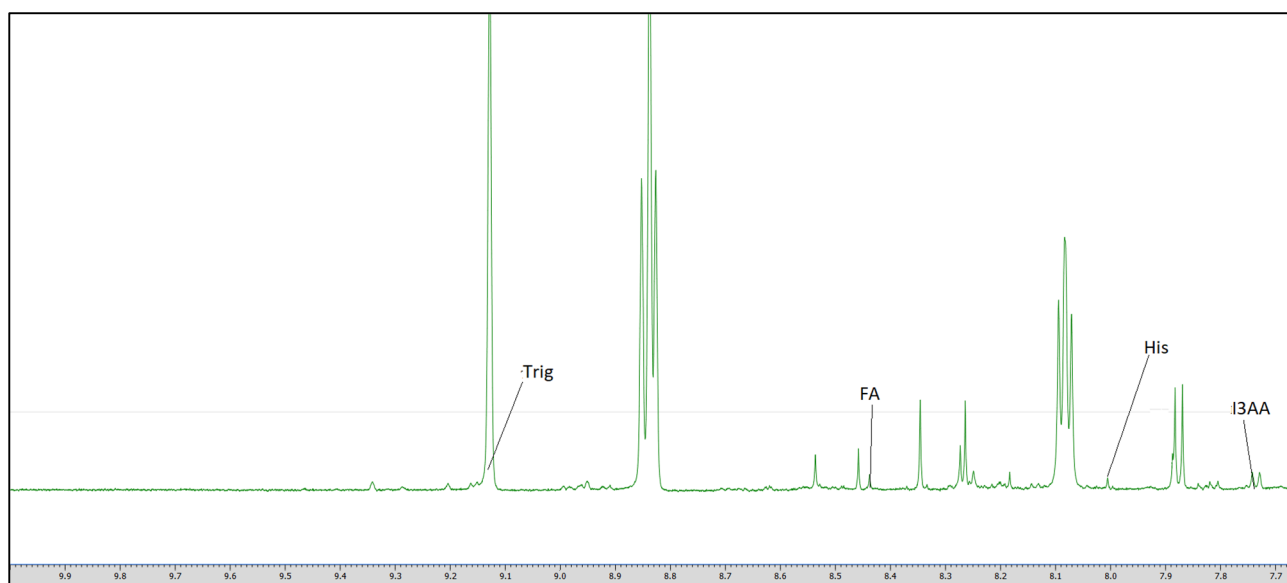


Figure S5: ^1H Spectrum of yellow pea hydroalcoholic extract, 7.7- 9 ppm. Molecule abbreviations are reported in Table S1.

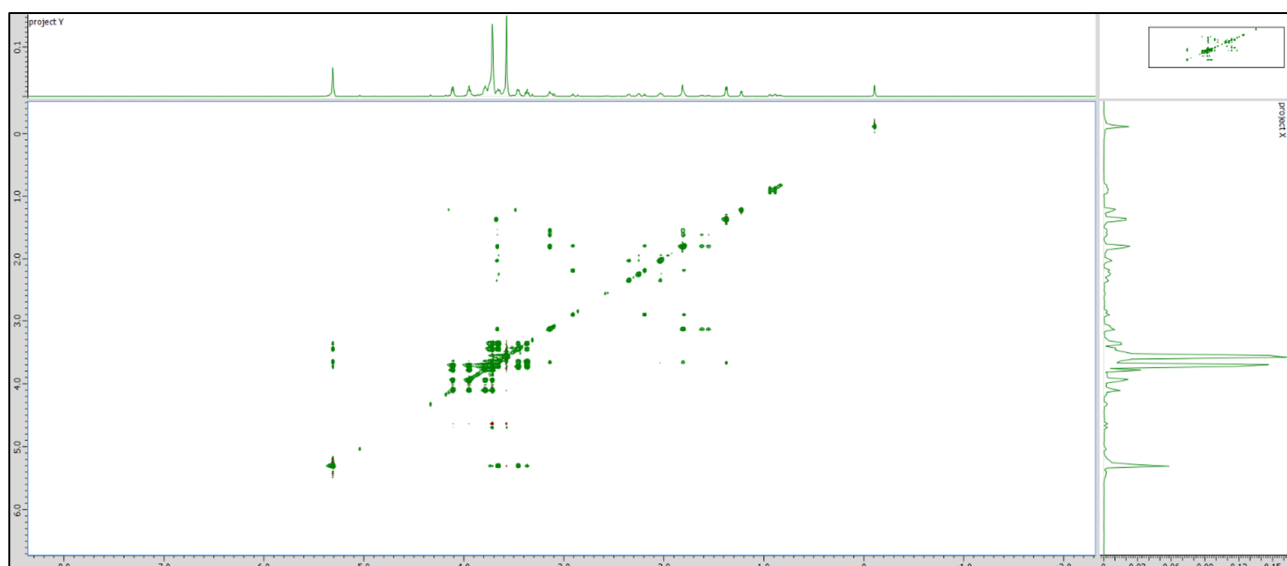


Figure S6: Bidimensional ^1H - ^1H TOCSY spectrum of yellow pea.

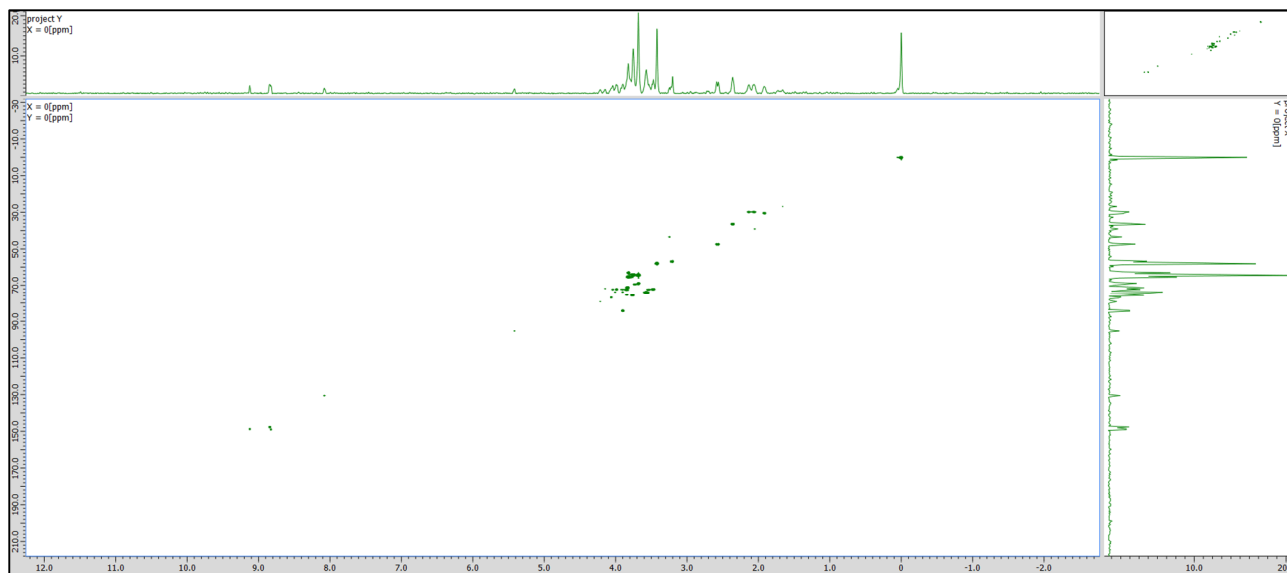


Figure S7: Bidimensional ^1H - ^{13}C HSQC spectrum of yellow pea.

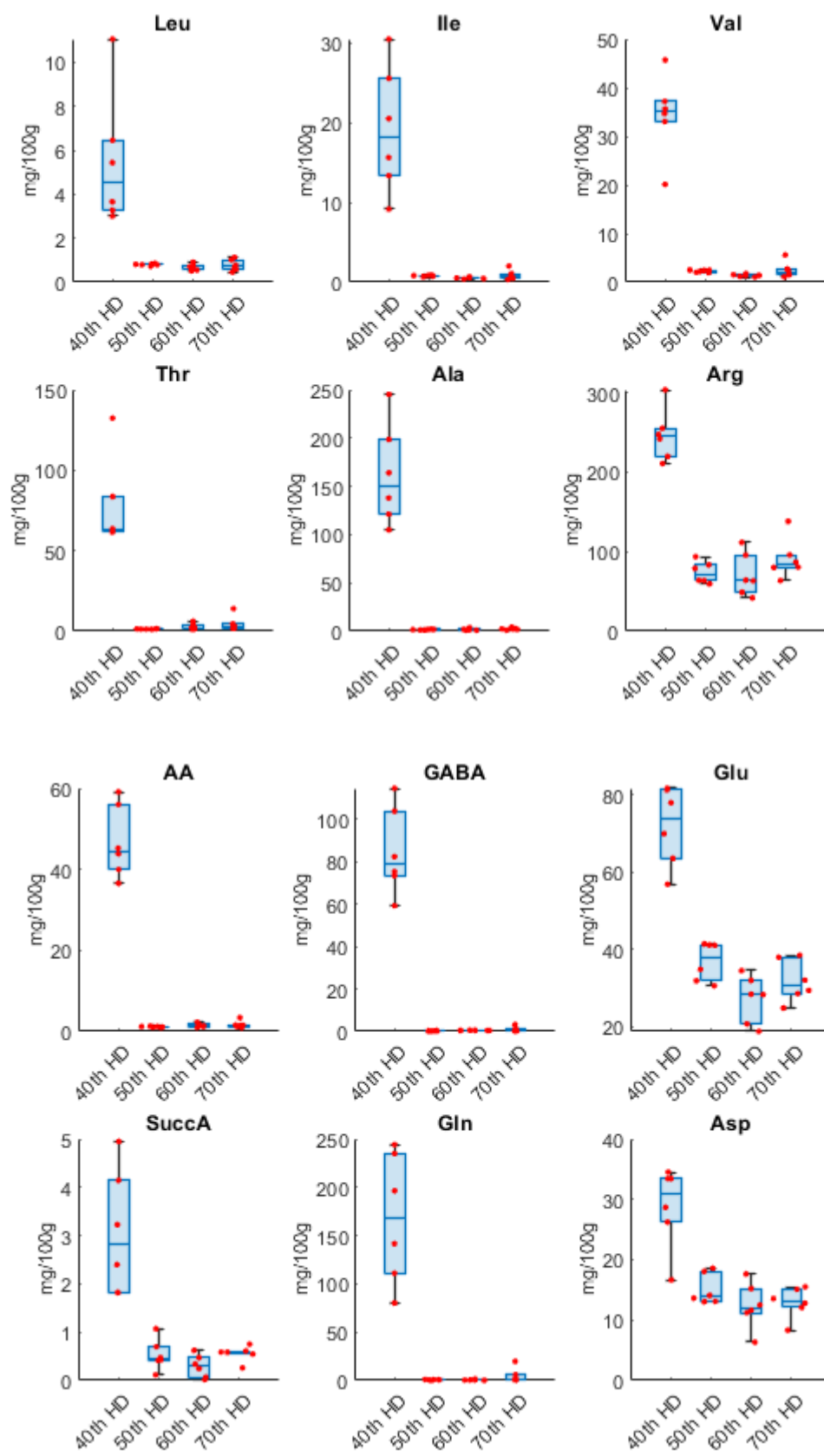
Compound	Assignment	^1H δ (ppm)	Multiplicity	^{13}C δ (ppm)
Organic Acid				
Acetic acid (AA)	CH ₃	1.92	s	25.98
Caffeic acid (CaffAc)	CH-2	7.55	d	117.15
	CH-5	7.03	dd	124.122
	CH-3	6.96	dd	118.85
	CH-4	6.80	dd	143.34
	CH-1	6.57	d	124.32
Hydroxycinnamic acid (4-HCA)	β -CH	7.57	d	144.6
	CH-2,4	7.40	m	130.2
	CH-1	6.79	m	116.13
	α -CH	6.50	d	117.9
Citric acid (CA)	α,γ -CH	2.67	d	44.77
	α',γ' -CH	2.71	d	44.77
Formic acid (FA)	CH	8.46	s	171.90
Fumaric acid (FumA)	CH=CH	6.51	s	137.94
Malic acid (MA)	α -CH	4.31	dd	69.33
	β,β' -CH	2.38,2.69	dd	40.86
p-Hydroxyphenylacetic acid (OHPheAc)	CH-1	6,96	m	178.29
	CH-2	6,78	m	168.90
	CH-3,4	6,78	m	159.41
	CH ₃	2,14	s	20.63
Propionic acid (PA)	CH ₂	2,26	q	28.21
	CH ₃	1,29	t	9.51
Succinic acid (SA)	α,β -CH ₂	2.42	s	36.31
Amino acids				
Alanine (Ala)	β -CH ₃	1.49	d	19.05
	α -CH	3.80	q	53.56
Asparagine (Asn)	β' -CH	2.86	dd	37.44
	β -CH	2.89	dd	37.44
	α -CH	4.01	m	54.09
Aspartate (Asp)	α -CH	3.89	t	55.09
	β -CH	2.96	dd	39.30
	β' -CH	2.66	dd	39.30
Arginine (Arg)	α -CH	3.76	t	57.26
	δ -CH ₂	3.23	t	43.32
	β -CH ₂	1.90	m	30.49
	γ -CH ₂	1.65	m	26.45
gamma-Aminobutyric acid (GABA)	γ -CH ₂	3.00	t	42.21
	α -CH ₂	2.28	t	37.05
	β -CH ₂	1.89	m	26.38
Glutamate (Glu)	γ -CH ₂	2.09	m	28.9
	β -CH ₂	2.34	m	36.32
	α -CH	3.74	m	56.79
Glutamine (Gln)	γ -CH ₂	2.11	m	57.23
	β -CH ₂	2.45	m	33.93
	α -CH	3.81	m	29.29

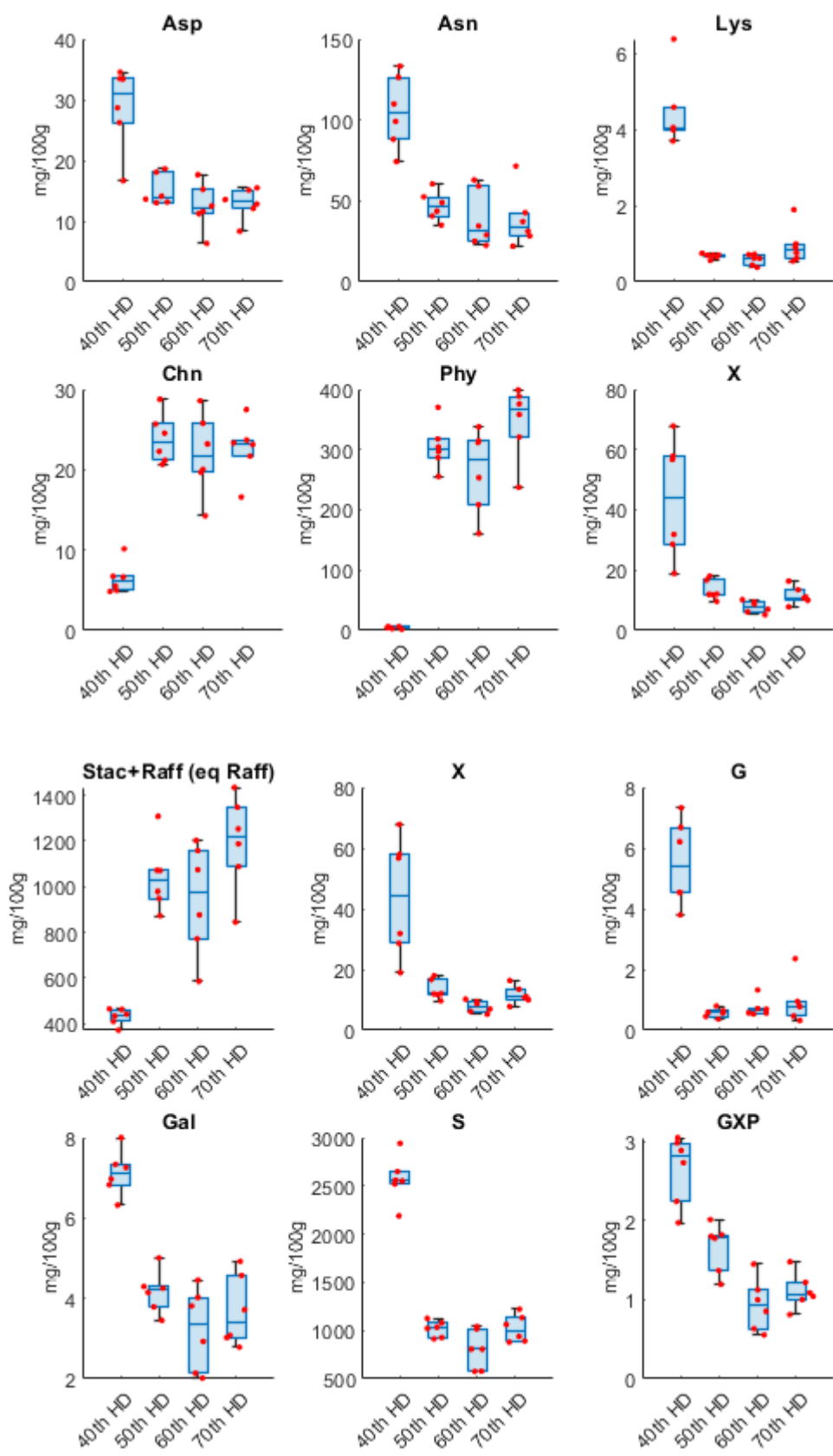
Histidine (His)	CH-6	3.19	dd	30.25
	CH-7	3.99	dd	57.29
	CH-5	7.09	d	120.03
	CH-2	8.01	d	138.31
Isoleucine (Ile)	δ -CH ₃	0.95	t	13.85
	γ-CH₃	1.02	d	17.38
	γ' -CH	1.25	m	27.01
	γ'' -CH	1.49	m	27.01
	β -CH	1.99	m	38.71
	α -CH	3.69	m	63.04
Leucine (Leu)	δ,δ'-CH₃	0.97	m	23.85, 24.59
	γ -CH	1.72	m	26.81
	β -CH ₂	1.73	m	42.60
	α -CH	3.74	m	56.21
Lysine (Lys)	δ -CH ₂	1.47	m	28.41
	γ -CH ₂	1.69	m	30.51
	β -CH ₂	1.95	m	43.27
	δ-CH₂	3.03	t	38.12
	α -CH ₂	3.78	t	57.32
Phenylalanine (Phe)	CH-2,6	7.32	d	130.3
	CH-4	7.38	d	128.6
	CH-3,5	7.42	d	130.3
	β -CH ₂	3.27	m	37.1
	α -CH	3.98	dd	56.8
Threonine (Thr)	γ-CH₃	1.32	d	22.15
	α -CH	3.60	m	63.46
	β -CH	4.27	m	68.94
Tryptophan (Trp)	CH-5	7.20	t	124.9
	CH-6	7.27	t	127.9
	CH-7	7.53	d	114.7
	CH-4	7.73	d	121.2
Tyrosine (Tyr)	CH-2,6	7.17	d	130.0
	CH-3,5	6.89	d	117.0
	β -CH ₂	3.15	dd	37.1
	α -CH	3.93	dd	56.8
Valine (Val)	γ -CH ₃	0.99	d	19.41
	γ'-CH₃	1.05	d	20.75
	β -CH	2.29	m	31.89
	α -CH	3.62	m	63.36
<u>Carbohydrates</u>				
Fructose (F)	CH-1	3.69	m	62.94
	C-2	\	\	104.23
	CH-3	4.22	d	77.24
	CH-4	4.06	m	75.04
	CH-5	3.90	m	83.37
	CH-6	3.82	m	63.88
α-Galactose (α-Gal)	CH-1	5.26	d	95.1
	CH-2	3.78	m	72.8
	CH-3	3.98	d	73.3
	CH-4	3.78	m	61.8
	CH-5	4.08	t	73.3
	CH6,6'	3.73	m	70.7
β-Galactose (β-Gal)	CH-1	4.59	d	97.3
	CH-2	3.50	dd	72.9
	CH-3	3.62	dd	73.8

	CH-4	3.97	d	69.7
	CH-5	3.72	m	76.1
	CH-6,6'	3.82	m	72.2
α -Xylose (α -X)	CH-1	5.14	d	93.1
	CH-6	3.80	m	72.5
	CH-3	3.70	m	73.8
	CH-5	3.45	m	70.7
	CH-2	3.35	m	72.5
	CH ₂ -6	3.73-3.90	m	96.9
β -Xylose (β -X)	CH-1	4.56	d	97.0
	CH-5	3.87	m	74.6
	CH-3	3.69	m	76.8
	CH-4	3.36	m	70.7
	CH-2	3.18	m	75.2
α -Glucose (α -G)	CH-1	5.23	d	93.10
	CH-2	3.55	m	72.49
	CH-3	3.72	m	73.84
	CH-4	3.42	m	70.67
	CH-5	3.84	m	72.52
	CH ₂ -6	3.73, 3.90	m	96.97
β -Glucose (β -G)	CH-1	4.69	d	96.97
	CH-2	3.26	m	75.17
	CH-3	3.50	m	76.84
	CH-4	3.42	m	70.70
	CH-5	3.48	m	74.57
	CH ₂ -6	3.74, 3.91	m	61.80
Raffinose (Raff)	GLC CH-1	5.45	d	102.4
	GAL CH-1	5.01	d	93.9
	FRU CH-3	4.22	d	63.6
Stachyose (Stach)	GLC CH-1	5.47	d	94.8
	GAL CH-1	5.01	bs	101.0
	FRU CH-3	4.22	d	79.8
Verbascose (Verb)	GLC CH-1	5.50	d	105.2
	GAL CH-1	5.02	bs	102.8
	FRU CH-3	4.22	d	78.3
Sucrose (S)	G CH-1	5.44	d	93.22
	CH-2	3.59	m	72.11
	CH-3	3.79	m	73.54
	CH-4	3.48	m	70.26
	CH-5	3.85	m	73.38
	CH ₂ -6	3.82	m	61.18
	F CH ₂ -1'	3.69	m	62.44
	C-2	\	\	104.85
	CH-3'	4.22	m	77.45
	CH-4'	4.06	m	75.04
	CH-5'	3.90	m	82.44
	CH ₂ -6	3.82	m	63.38
Miscellaneous Metabolites				
Phytate (Pht)	CH-1	4.15	dd	74.93
	CH-2,2'	3.61	m	75.13
	CH-3,3'	3.53	m	73.97
	CH-4	3.27	dd	76.98
Uracil	CH	5.80	d	103.71
	CH	7.52	d	146.30

Uridine (U)	CH-2	7.86	d	144.6
	CH-3	5.90	d	92.1
	CH-1	5.86	d	105.1
	CH-6	4.34	dd	76.5
	CH-5	4.22	t	72.1
	CH-4	4.12	m	87.1
	CH-7	3.91	dd	63.6
	CH-7'	3.80	dd	63.6
Uridine n-phosphate (UXP)	URA CH-5 ring	5.97	d	105.23
	CH-6 ring	7.89	d	144.40
	RIB CH-1	5.98	d	91.29
Guanosine n-phosphate (GXP)	CH-17'	4.18	m	67.40
	CH-17	4.24	m	67.40
	CH-5	4.36	m	73.10
	CH-4	4.58	dd	76.35
	CH-2	6.04	d	89.33
	CH-7	8.12	s	140.39
Rutin- Glycoside (Rut)	CH 2,6	6.39	d	104.94
	CH 4	6.21	t	101.97
	CH 2',6'	7.64	d	128.13
	CH 3',5'	6.88	d	117.58
	Glucose CH1	4.52	d	100.72
Indole-3-Acetic Acid (I3AA)	CH-2	7.21	s	124.7
	CH-4	7.33	d	112.2
	CH-5	7.08	t	121.9
	CH-6	7.01	t	119.7
	CH-7	7.73	d	119.8
	CH-8	3.65	s	37.2
Adenosine phosphate (AXP)	CH-17'	4.18	ddd	67.40
	CH-17	4.24	ddd	67.40
	CH-5	4.36	m	73.10
	CH-4	4.58	dd	76.35
	CH-2	6.14	d	89.33
	CH-7	8.12	s	140.39
Choline (Chn)	N-(CH₃)₃	3.21	s	56.74
	CH ₂	3.51	t	70.20
	CH ₂	4.07	t	57.78
Trigonelline (Trg)	CH-1	9.11	s	148.4
	CH-3	8.83	m	148.71
	CH-4	8.11	m	130.58
	CH-5	8.83	m	148.71
	CH ₃	4.43	s	51.07

Table S1. Metabolites identified in the ¹H NMR spectrum of the aqueous extracts of yellow peas. In bold are evidenced the resonances chosen for metabolite quantification; s: singlet, bs: broad singlet, d: doublet, t: triplet, q: quadruplet, dd: doublet of doublets, m: multiplet.





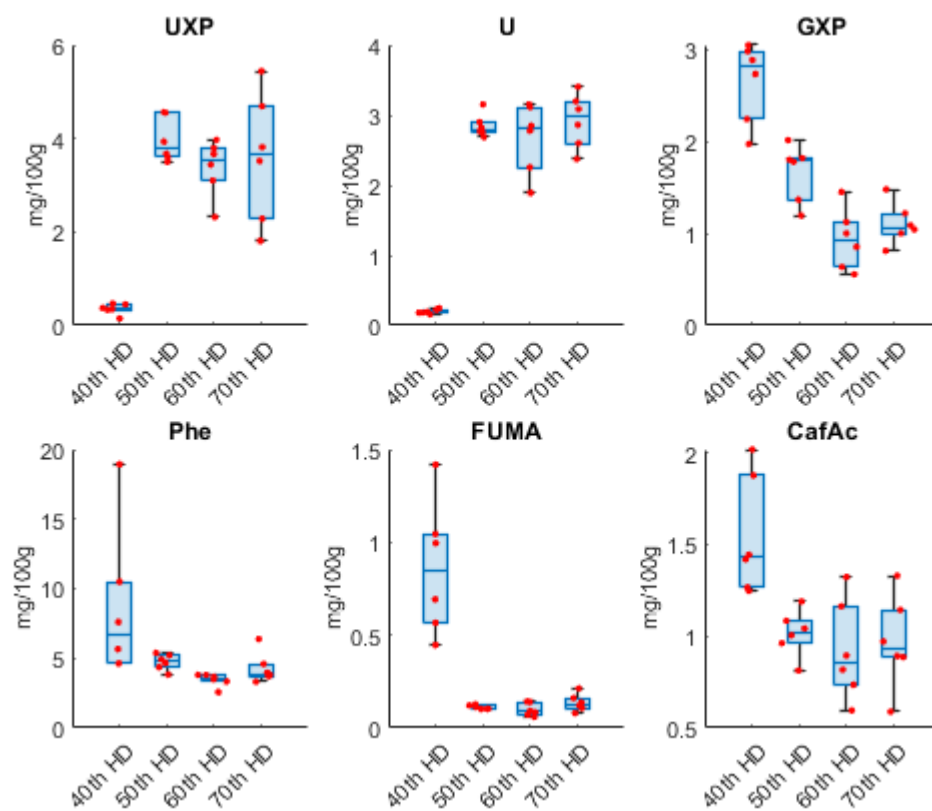


Figure S8: Boxplot of significant variables ($p < 0.05$) in ANOVA one-way test

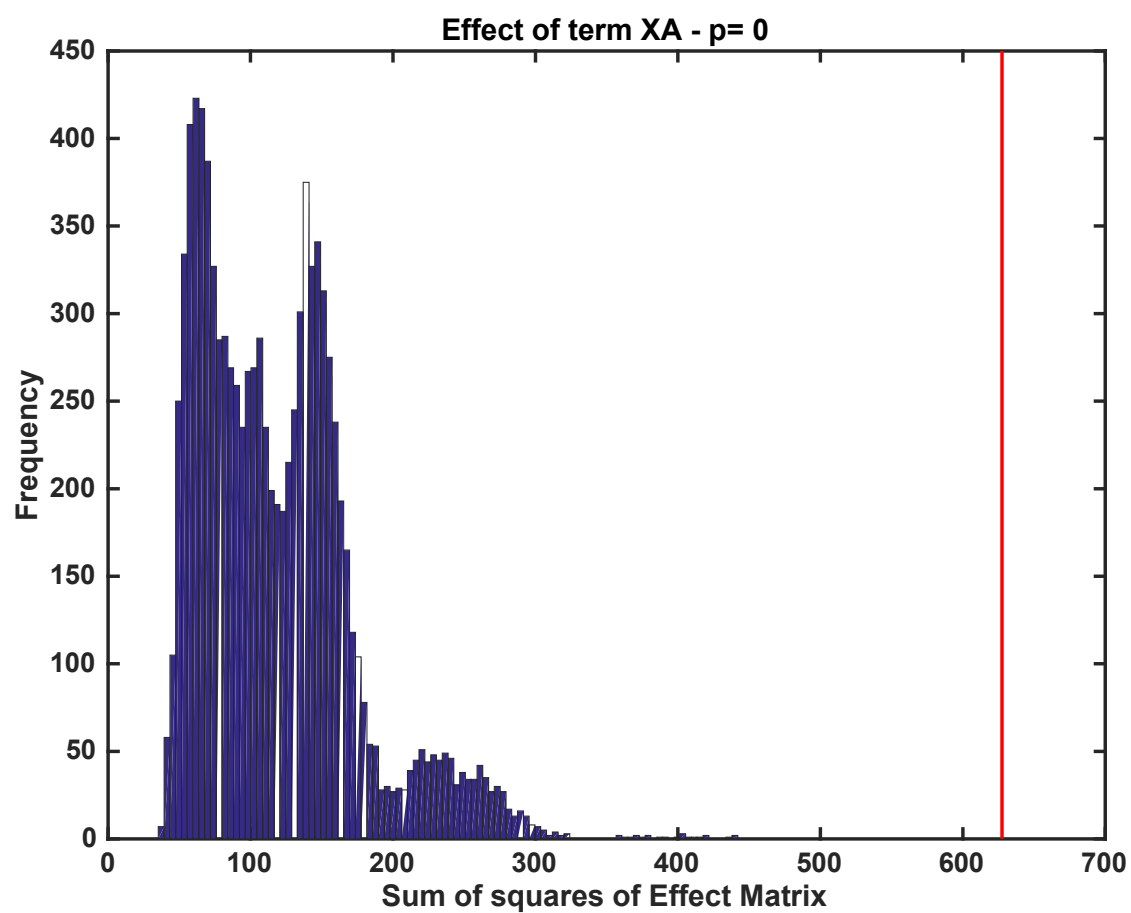


Figure S9: ASCA Permutation Test for Time factor X_A

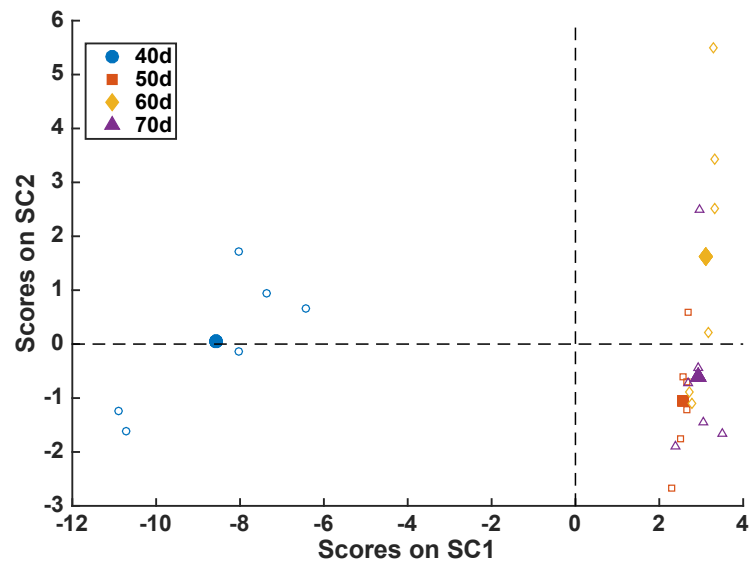


Figure S10: ASCA Scores plot Scatterplot SC1 and SC2 for time factor X_A .

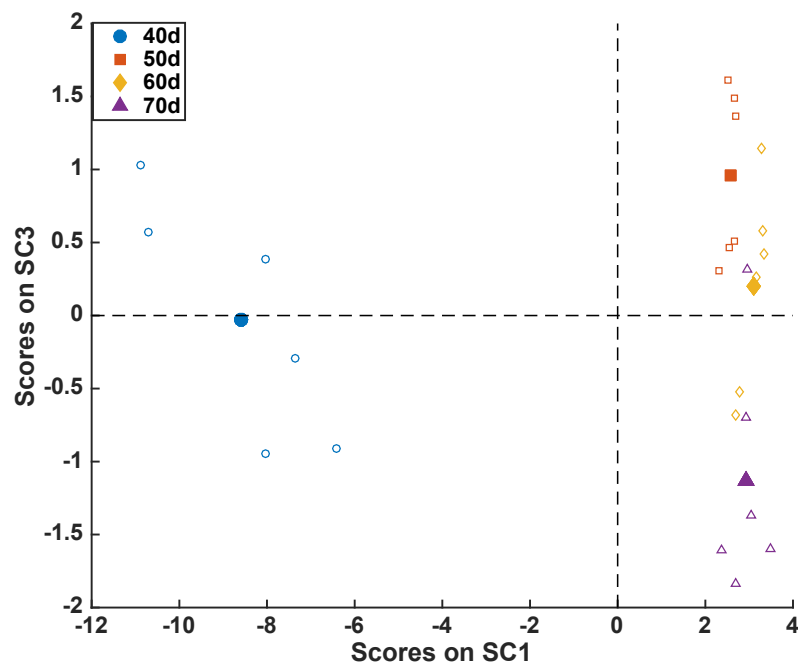


Figure S11: ASCA Scores plot Scatterplot for SC1 and SC3 for time factor X_A .

Figure S12: Spearman's correlation Heatmap, 'Eso' cultivar, 40th day of harvest.

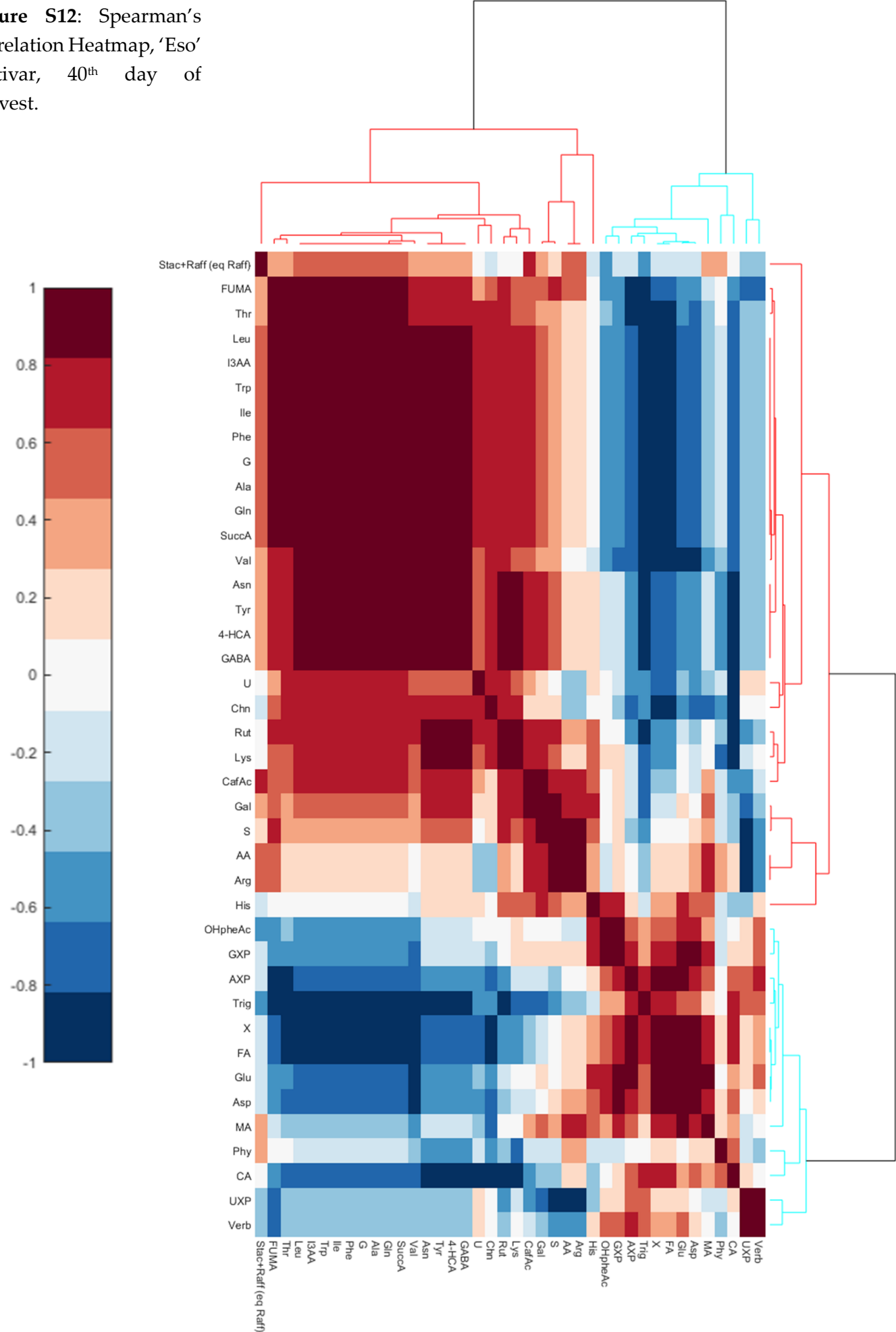


Figure S13:
Spearman's
correlation Heatmap,
'Eso' cultivar, 50th day
of harvest.

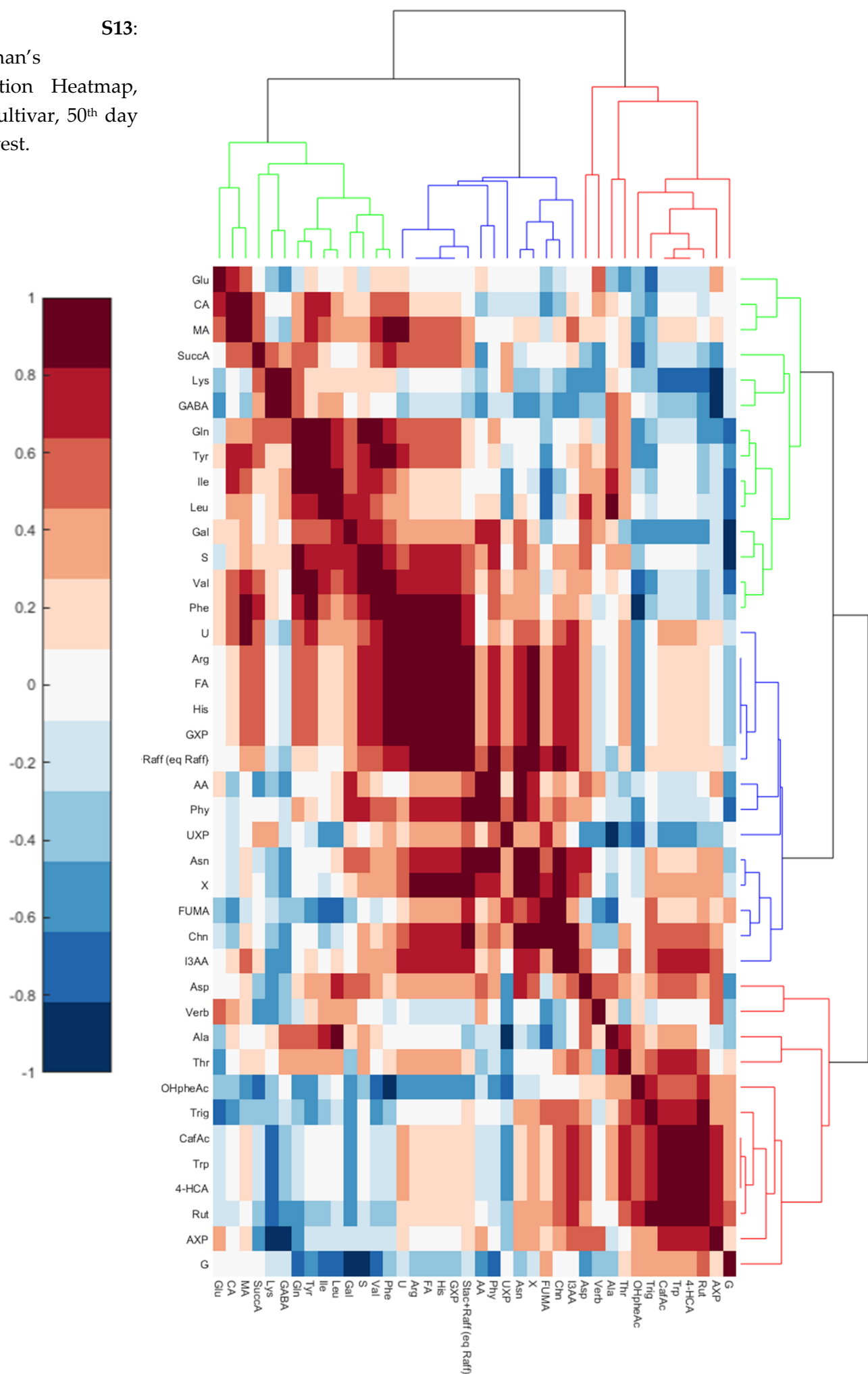


Figure S14:
Spearman's
correlation
Heatmap, 'Eso'
cultivar, 60th day
of harvest.

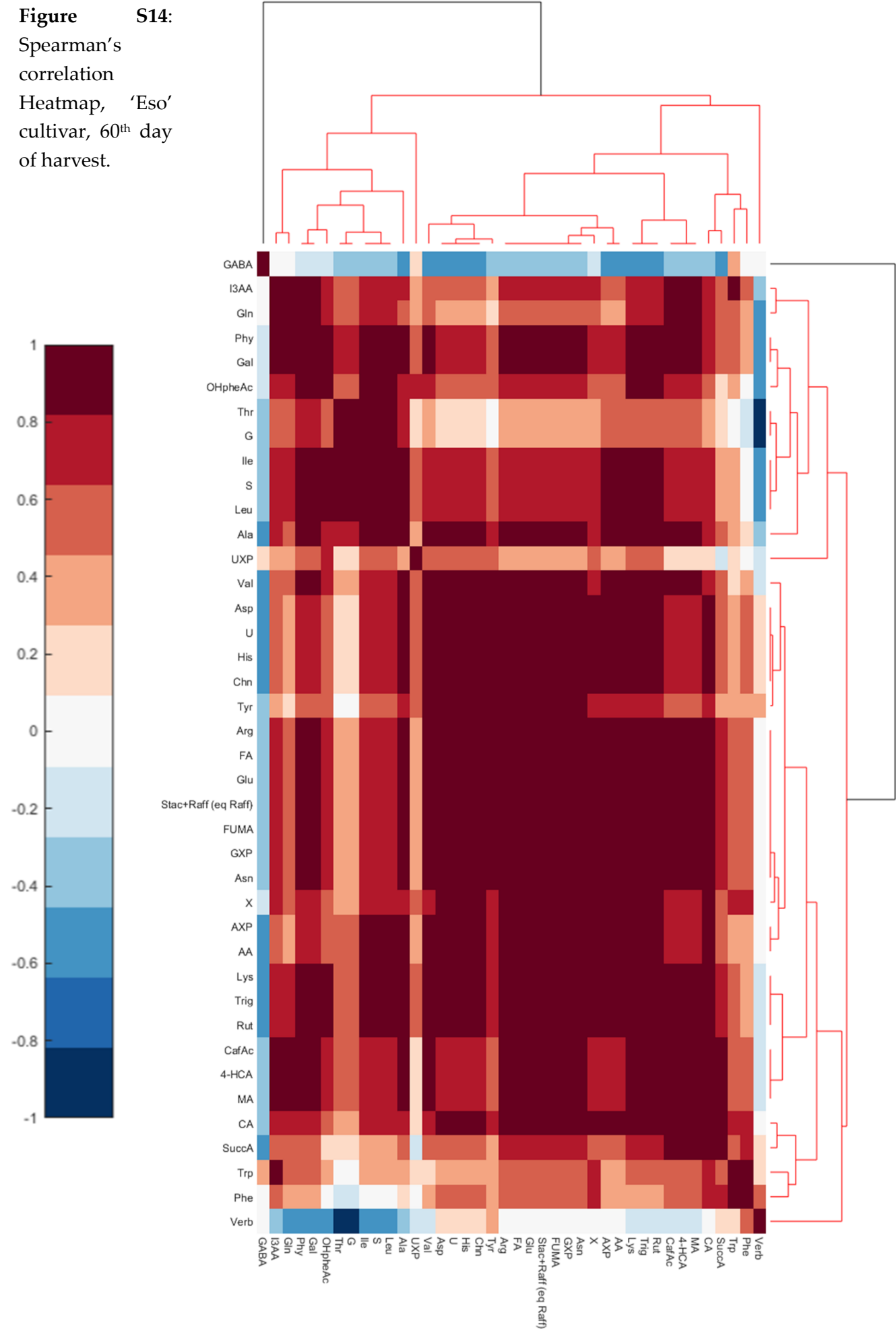


Figure S15:
Spearman's
correlation
Heatmap, 'Eso'
cultivar, 70th day
of harvest.

